REMARKS

Status of the Claims

Claims 1-66 are currently pending in the above-referenced patent application; claims 55, 64, 65, and 66 were previously amended.

In the Office Action, the claims are rejected, in various combinations, under 35 U.S.C. § 102(b) as allegedly anticipated and under 35 U.S.C. § 103(a) as allegedly obvious. For the reasons set forth below, each of these rejections is overcome.

The Invention

The present invention is directed to liposome compositions comprising a lipid and a condensing agent-nucleic acid complex encapsulated within the liposome.

Prior Interview

During the discussions of October 13, 2004 and October 14, 2004, between Examiner Kishore and Applicants' representatives Eugenia Garrett-Wackowski and Carol Fang, Applicants pointed out that the present claims are directed to liposomal formulations comprising a condensing agent-nucleic acid complex that is ***encapsulated*** in a liposome. During the discussions, the Examiner agreed that nucleic acid-lipid complexes do not anticipate or render obvious liposomes encapsulating nucleic acid-condensing agent complexes.

Rejections Under 35 U.S.C. § 102(b)

The claims have been rejected, in various combinations, under 35 U.S.C. § 102(a) over a number of different references. In response, Applicants respectfully traverse each of the §102 anticipation rejections.

As set forth in MPEP § 2131, anticipation under § 102 can be found only when a cited reference discloses ***all*** of the elements, features or limitations of the presently claimed invention. The Examiner must establish that a single prior art reference discloses each and every element of the claimed invention. *See, e.g., Hybritech Inc. v. Monoclonal Antibodies, Inc.*, 231 U.S.P.Q. 81 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987).

1. Rejection of claims 1-8, 12-13, 15-17, 21-22, 32-39, 43-45, 49, 55, 57, and 59 as allegedly anticipated by U.S. Patent No. 5,908,777 ("Lee *et al.*")

The Examiner alleges that Lee *et al.* anticipates the presently claimed invention because the reference allegedly discloses liposomal compositions containing a nucleic acid wherein the nucleic acid is in a complex formed with a histone.

As explained above and by Dr. MacLachlan in his declaration under 37 C.F.R. § 1.132 (*see*, Declaration ¶ 7), the present invention is directed to liposomal formulations comprising a lipid and a condensing agent-nucleic acid complex encapsulated within the liposome.

As explained by Dr. MacLachlan, Lee *et al.* discloses nucleic acid-lipid ***complexes*** comprising anionic liposomes and nucleic acid-polylysine complexes formed by mixing preformed liposomes with nucleic acid-polylysine complexes in deionized water (*see*, Declaration ¶¶ 9). As Dr. MacLachlan clarifies, given that DNA does not readily cross lipid membranes, one of skill in the art would appreciate that mixing of a nucleic acid-polylysine complex with preformed liposomes in an aqueous solution does not result in entrapment of DNA within the internal space of the liposomes, but would, instead, result in formation of nucleic acid-lipid ***complexes*** (*see*, Declaration ¶ 9). Without a step that destabilizes the liposome membrane, the nucleic acid would not be able to enter the liposome and be encapsulated (*see,* Declaration ¶ 9). Thus, in contrast to the presently claimed liposomes, the nucleic acid-lipid complexes of Lee *et al.* do not comprise a nucleic acid fully encapsulated in a liposome (*see*, Declaration ¶¶ 9 and 18) and Lee *et al.* does not anticipate the presently claimed invention.

Accordingly, Applicants urge the Examiner to withdraw this aspect of the rejection under 35 U.S.C. § 102(a).

2. Rejection of claims 1-6, 12-13, 15-17, 21-22, 28, 32-37, 39, 43-45, 49, 55, 57, and 59 as allegedly anticipated by U.S. Patent No. 5,891,468 ("Martin")

The Examiner has also alleged that Martin anticipates the presently claimed invention because the reference discloses liposomal formulations containing nucleic acid

complexes, wherein the nucleic acid is reacted with an organic polycation to produce a condensed nucleic acid.

As explained by Dr. MacLachlan, Martin discloses ***complexes*** formed by mixing preformed liposomes with plasmid-histone complexes (*see*, Declaration ¶ 10). Thus, as Dr. MacLachlan has clarified, Martin does not describe nucleic acid-histone complexes fully encapsulated in a liposome (*see*, Declaration ¶ 10).

Dr. MacLachlan further clarifies that the dehydration-rehydration-extrusion methods described in Martin cannot be used to encapsulate nucleic acids (*see*, Declaration ¶¶ 10 and 16). Specifically, Dr. MacLachlan describes experiments conducted under his supervision that use dehydration-rehydration-extrusion methods to attempt to encapsulate nucleic acids in liposomes (*see*, Declaration ¶ 16). The experiment described by Dr. MacLachlan were conducted as follows. First a lipid solution containing a total of 2.22 μmoles lipid and comprising DOPE:DODAC:PEG-ceramide C14 (82.5:7.5:10 molar percent) was prepared by dissolving the lipids in chloroform and using nitrogen gas to drive off chloroform to form a lipid film. The lipid film was then hydrated with 2 ml phosphate buffered saline (pH 7.4) containing 50 or 100 μg of nucleic acid (*i.e.*, plasmid DNA) to generate liposomal samples with drug (*i.e.*, nucleic acid):lipid ratios of 22.5 and 45 μg input DNA/μmol lipid. The resulting suspension was subjected to 5 rounds of freezing in liquid nitrogen and thawing in a 37°C water bath, to increase homogeneity of the resulting multilamellar vesicles which were all greater than 10,000 nm in diameter. To produce liposomes of appropriate size, the samples were then extruded 10 times through 2 stacked 100 nm polycarbonate filters using a 10-mL Extruder (Northern Lipids Inc.) and nitrogen gas at 400-600 psi. Nucleic acid encapsulation was determined using membrane-impermeable Picogreen which fluoresces in the presence of plasmid DNA. The proportion of nucleic acid encapsulated in the liposomes was determined by measuring the fluorescence intensity of the Picogreen before and after the addition of the detergent Triton X-100 (*see*, Declaration ¶ 16).

The results from the experiments are set forth in Exhibit B accompanying Dr. MacLachlan's Declaration and demonstrate that plasmid encapsulation and recovery were both extremely inefficient at both of the input nucleic acid amounts examined. Specifically, as Dr.

MacLachlan explains, prior to extrusion, only 12% or 15% of the input nucleic acid was inaccessible to Picogreen due to its association with or incorporation into >10,000 nm multilamellar vesicles (*see*, Declaration ¶ 16 and Exhibit B). In addition, only 1.4% or 2% of the input nucleic acid was actually recovered after the extrusion step necessary to form actual liposomes (*see*, Declaration ¶ 16 and Exhibit B). Furthermore, only 0.055% or 0.14% of the input nucleic acid was recovered and encapsulated post extrusion (*see*, Declaration ¶ 16 and Exhibit B). Particle sizes for all of these extruded samples were all considerably larger than 100 nm (*see*, Declaration ¶ 16). As Dr. MacLachlan confirms, these results unequivocally demonstrate that the dehydration-rehydration-extrusion methods set forth in Martin do ***not*** produce liposomes that encapsulate plasmid DNA (*see*, Declaration ¶¶ 16-17). Thus, Martin does not anticipate the presently claimed liposomes encapsulating a nucleic acid-condensing agent complex.

Accordingly, Applicants urge the Examiner to withdraw this aspect of the rejection under 35 U.S.C. § 102(a).

2. Rejection of claims 1-8, 12-13, 15-17, 21-22, 32-39, 43-45, 49, 55, 57, and 59 as allegedly anticipated by Lee *et al.*, *J. Biol. Chem.* 271(14):8481-8487 (1996) ("Lee *et al.* 2")

The Examiner also alleges that Lee *et al.* 2 anticipates the presently claimed invention because the reference allegedly discloses liposomal formulations containing condensed nucleic acid complexes.

As explained by Dr. MacLachlan, Lee *et al.* 2 discloses nucleic acid-liposome ***complexes*** that are the same as or similar to the complexes disclosed in Lee *et al.* (*see*, Declaration ¶ 11). Specifically, Lee *et al.* describes lipoplexes, *i.e.*, ***complexes*** between the liposomes and nucleic acid-condensing agent which are formed by mixing preformed anionic liposomes with nucleic acid-polylysine complexes in deionized water (*see,* Declaration ¶ 11). As discussed above in connection with the rejection of the claims as allegedly anticipated by Lee *et al.* and as explained by Dr. MacLachlan, one of skill in the art would appreciate that mixing preformed liposomes with nucleic acid-polylysine complexes in an aqueous solution would result in formation of lipoplexes, and not liposomes fully encapsulating a nucleic acid (*see,*

Declaration ¶ 11). Thus, in contrast to the presently claimed liposomes, the nucleic acid-lipid complexes of Lee *et al.* 2 also do not comprise a nucleic acid fully encapsulated in a liposome and Lee *et al.* 2 does not anticipate the presently claimed invention.

In view of the foregoing statements, Applicants urge the Examiner to withdraw all of the rejections under 35 U.S.C. § 102(a).

Rejections under 35 U.S.C. § 103(a)

The claims have been rejected, in various combinations, under 35 U.S.C. § 103(a) over a number of different references. In response, Applicants respectfully traverse each of the §103 obviousness rejections.

As set forth in M.P.E.P. § 2143, to establish a *prima facie* case of obviousness, *three* basic criteria must be met: (1) there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings; (2) there must be a reasonable expectation of success; and (3) the prior art reference (or references when combined) must teach or suggest all the claim limitations.

All three elements set forth above must be present in order to establish a *prima facie* case of obviousness. As explained herein below in connection with each of the § 103(a) obviousness rejections, Applicants assert that a *prima facie* case of obviousness has not been established for at least the following reason: the cited art references do not teach or suggest all the claim limitations.

1. Rejection of claims 11-14, 26-28, 30-31, 42, 52-53, 56, 578, and 62-63 as allegedly obvious over Lee *et al.* or Lee *et al.* 2

In making this rejection, the Examiner acknowledged that neither Lee *et al.* nor Lee *et al.* 2 teach or suggest diameters of the condensing agent-nucleic acid complex or the addition of the condensing agent in stages or the addition of two condensing agents, but concludes that each of these parameters would be obvious in view of Lee *et al.* or Lee *et al.* 2. However, as discussed above in connection with the rejections of the claims under 35 U.S.C. § 102(a) and as explained by Dr. MacLachlan, neither Lee *et al.* nor Lee *et al.* 2 disclose or even

suggest the presently claimed liposomes encapsulating a nucleic acid-condensing agent complex (*see*, Declaration ¶¶ 8, 9, 11 and 18). Absent such a teaching or suggestion, the compositions and methods of the presently claimed invention are nonobvious, and thus patentable over Lee *et al.* or Lee *et al.* 2. Accordingly, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103.

2. Rejection of claims 17-22, 28-29, 45-48, 53-54, 60, and 63-64 as allegedly obvious over Lee *et al.* or Lee *et al.* 2 or Martin further in view of U.S. Patent No. 5,885,613 ("Holland")

In making this rejection, the Examiner acknowledges that none of Lee *et al.*, Lee *et al.* 2, or Martin disclose PEG-ceramide, but cites Holland as disclosing liposomal formulations comprising PEG-ceramide, and concludes that the presently claimed liposomal compositions would have been obvious over Lee *et al.*, Lee *et al.* 2, or Martin further in view of Holland *et al.*

As discussed in detail above and explained by Dr. MacLachlan, the presently claimed invention is directed to compositions comprising a nucleic-acid-condensing agent complex ***encapsulated*** in a liposome (*see*, Declaration ¶ 7). In contrast to the presently claimed invention, the disclosures of Lee *et al.*, Lee *et al.* 2, or Martin each disclose nucleic-acid lipid ***complexes*** (*see*, Declaration ¶¶ 9-11). Holland's disclosure of PEG-ceramide does not remedy the defect in either of the cited references. As explained by Dr. MacLachlan, Holland discloses the use of PEG-ceramide in a nucleic acid lipid ***complex*** (*see*, Declaration ¶ 13). More particularly, Holland *et al.* states:

> Cationic lipids have been used in the transfection of cells in vitro and in vivo. . . .The efficiency of this transfection has often been less than desired, for various reasons. ***One is the tendency for cationic lipids complexed to nucleic acid to form unsatisfactory carriers. These carriers are improved by the inclusion of PEG lipids.***
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> See, column 12, lines 28-39 of Holland et al. (emphasis added).

Thus, the teachings of Holland are clearly directed to forming nucleic acid-cationic liposome ***complexes***, which are structurally and functionally different from the presently claimed

liposomes, wherein the nucleic acid-condensing agent complex is encapsulated in the liposome is resistant in aqueous solution to degradation with a nuclease (*see*, Declaration ¶ 13). Moreover, as Dr. MacLachlan has explained, the dehydration-rehydration-extrusion methods set forth in Holland cannot be used to encapsulate a nucleic acid in a liposome (*see*, Declaration ¶¶ 16-17). Thus, the cited references alone, or in combination, do not teach or suggest the presently claimed liposomes ***encapsulating*** a condensing agent-nucleic acid complex. Absent such a teaching or suggestion, the compositions and methods of the presently claimed invention are nonobvious, and thus patentable over Lee *et al.*, Lee *et al.* 2, or Martin further in view of Holland.

Accordingly, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103.

3. Rejection of claims 8-10, 23-25, 39-40, 50-51, and 61 as allegedly obvious over Lee *et al.* or Lee *et al.* 2 or Martin further in view of U.S. Patent No. 6,420,176 ("Lisziewicz").

In making this rejection, the Examiner acknowledges that none of Lee *et al.*, Lee *et al.* 2, Martin disclose the use of polythethylenimine, but cites Lisziewicz *et al.* as disclosing polyethylenimine as a nucleic acid condensing agent, and concludes that the presently claimed liposomal compositions would have been obvious over Lee *et al.* or Lee *et al.* 2 or Martin further in view of Lisziewicz *et al.*

As discussed in detail above and explained by Dr. MacLachlan, the presently claimed invention is directed to compositions comprising a nucleic-acid-condensing agent complex ***encapsulated*** in a liposome (*see*, Declaration ¶ 7). In contrast to the presently claimed invention, the disclosures of Lee *et al.*, Lee *et al.* 2, or Martin each disclose nucleic-acid lipid ***complexes*** (*see*, Declaration ¶¶ 9-11) . As explained by Dr. MacLachlan, Lisziewicz's disclosure of polyethylenimine (PEI) does not remedy the defect in any of the cited references (*see*, Declaration ¶ 14). If anything, Lisziewicz teaches away from the use of PEI. Specifically, as Dr. MacLachlan clarifies, Lisziewicz compares the efficiency and toxicity of PEI and PEI-mannose as a condensing agent and demonstrates that relative to PEI mannose, PEI (1) is more toxic; (2) requires more DNA to neutralize; and (3) is less efficient for transfection (*see*, Declaration ¶ 14). Thus, one of skill in the art would not have been motivated to use PEI in view

of the disclosure of Lisziewicz. Even if Lee *et al.* or Lee *et al.* 2 or Martin were combined with Lisziewicz, the combination would not lead to the presently claimed invention because none of the cited references alone, or in combination, teach or suggest condensing agent-nucleic acid complexes ***encapsulated*** in a liposome. Absent such a teaching or suggestion, the compositions and methods of the presently claimed invention are nonobvious, and thus patentable over Lee *et al.* or Lee *et al.* 2 or Martin in view of Lisziewicz.

Accordingly, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103.

4. Rejection of claims 65-66 as allegedly obvious over Lee *et al.* or Lee *et al.* 2 or Martin in combination with WO 98/20857 ("Papahadjopoulos").

In making this rejection, the Examiner acknowledges that none of Lee *et al.*, Lee *et al.* 2, Martin disclose the use of reverse phase evaporation or detergent dialysis to prepare liposomes, but alleges that Papahadjopoulos describes such methods for making liposomes.

As discussed in detail above and explained by Dr. MacLachlan, the presently claimed invention is directed to compositions comprising a nucleic-acid-condensing agent complex ***encapsulated*** in a liposome (*see*, Declaration ¶ 7). In contrast to the presently claimed invention, the disclosures of Lee *et al.*, Lee *et al.* 2 or Martin each disclose nucleic-acid lipid ***complexes*** (*see*, Declaration ¶¶ 9-11) . Moreover, as explained by Dr. MacLachlan, Papahadjopoulos does not remedy the defect in any of the cited references (*see*, Declaration ¶ 15). Papahadjopoulos discloses nucleic acid-lipid ***complexes*** formed by mixing preformed liposomes with nucleic acids which leads to formation of lipoplexes, *i.e.*, complexes between the nucleic acids and liposomes, and will ***not*** lead to encapsulation of the nucleic acid in the liposomes (*see*, Declaration ¶¶ 9 and 15). In fact, the disclosure of Papahadjopoulos explicitly states that the methods described therein are used for forming ***complexes*** between preformed liposomes and nucleic acids and does not disclose or suggest encapsulating nucleic acids in liposomes using detergent dialysis or reverse phase evaporation (*see*, Declaration ¶ 15). Thus, the cited references alone, or in combination, do not teach or suggest condensing agent-nucleic acid complex ***encapsulated*** in a liposome. Absent such a teaching or suggestion, the compositions and

methods of the presently claimed invention are nonobvious, and thus patentable over Lee *et al.* or Lee *et al.* 2 or Martin in combination with Papahadjopoulos.

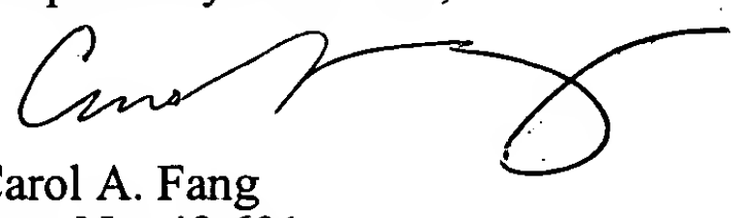
Accordingly, Applicants respectfully request withdrawal of this rejection under 35 U.S.C. § 103.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at 925-472-5000.

Respectfully submitted,



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